Presymptomatic spinal cord pathology in c9orf72 mutation carriers: a longitudinal neuroimaging study

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Abstract

Objective: *C9orf72* hexanucleotide repeats expansions account for almost half of familial Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD) cases. Recent imaging studies in asymptomatic *c9orf72* carriers have demonstrated cerebral white (WM) and grey matter (GM) degeneration before the age of 40.

The objective of this study was to characterise cervical spinal cord (SC) changes in asymptomatic *c9orf72* hexanucleotide carriers.

Methods: Seventy-two asymptomatic individuals were enrolled in a prospective study of first-degree relatives of ALS and FTD patients carrying the *c9orf72* hexanucleotide expansion. Forty of them carried the pathogenic mutation (C9+). Each subject underwent quantitative cervical cord imaging. Structural grey (GM) and white matter (WM) metrics and diffusivity parameters were evaluated at baseline and 18 months later. Data were analysed in C9+ and C9- subgroups and C9+ subjects were further stratified by age.

Results: At baseline, significant WM atrophy was detected at each cervical vertebral level in C9+ subjects older than 40 without associated changes in GM and DTI parameters. At 18-month follow-up, WM atrophy was accompanied by significant corticospinal tract (CST) fractional anisotropy (FA) reductions. Intriguingly, asymptomatic C9+ subjects older than 40 with family history of ALS (as opposed to FTD) also exhibited significant CST FA reduction at baseline.

Discussion: Cervical SC imaging detects WM atrophy exclusively in C9+ subjects older than 40 years of age and progressive CST FA reductions can be identified on 18-month follow-up. Cervical SC MRI readily captures presymptomatic pathological changes and disease propagation in *c9orf72*-associated conditions.

Keywords: *c9orf72*, presymptomatic carriers, spinal cord MRI, multi-parametric MRI, presymtomatic degeneration

Introduction

Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD) are adult-onset neurodegenerative conditions with shared pathological, genetic, neuropsychological and imaging features[1,2]. Up to 40% of familial ALS and FTD cases are caused by autosomal dominant GGGGCC hexanucleotide expansions in the non-coding region of the *c9orf72* gene[3,4]. While the minimum pathogenic number of hexanucleotide repeats remains to be established, and intermediate repeat numbers may also be pathological[5], most healthy individuals have no more than 23-30 repeats[6]. Despite the high penetrance of the mutation (up to 90%) which may increase with advancing age[7], the relationship between repeat numbers and age of symptom onset is not firmly established[8] and no validated prognostic indicators currently exist to predict the clinical manifestation of GGGGCC repeat expansions as FTD or ALS at the asymptomatic stage.

Expansions in the *c9orf72* gene can manifest in gain or loss of function[9] and lead to abnormal neuronal aggregates of nuclear RNA foci, dipeptides repeats (DPR) and TDP-43 inclusions[9]. RNA foci are widely distributed across the central nervous system (CNS) and can be observed within neuronal nuclei especially in the frontal and motor cortex, hippocampus, cerebellum and spinal cord (SC)[10]. DPR inclusions are positive for the ubiquitin-binding protein p62 and negative for TDP-43. They are commonly observed in the cerebellum, hippocampus and in the neocortex[11]. TDP-43 inclusions have been identified in frontal, temporal and primary motor regions, as well as in the hippocampus, basal ganglia, amygdala, thalamus and in the midbrain[10].

ALS studies have consistently demonstrated that considerable white matter (WM) and significant grey matter (GM) degeneration has already taken place by the time of the diagnosis, limiting the efficacy of neuroprotective interventions[12]. Asymptomatic carriers of disease-causing genetic mutations represent an optimal study population to characterise early pathological changes, anatomical patterns of propagation and mechanisms of disease spread. More importantly, the presymptomatic phase of the disease provides an invaluable opportunity to widen the therapeutic window and introduce neuroprotective therapy at the time when GM and WM integrity is still relatively preserved. This notion is increasingly relevant with the emergence of gene-targeted strategies for *c9orf72* associated conditions[13]. To date, the majority of presymptomatic *c9orf72* studies focused on cerebral imaging and captured GM and WM alterations years before expected symptom onset[14,15,16,17,18]. Recent studies indicate that widespread cortical and subcortical abnormalities can be detected in asymptomatic *c9orf72* carriers younger than 40 years of age[19]. The frontotemporal, insular, hippocampal and thalamic changes identified in asymptomatic cohorts are consistent with the genotype-associated signature of symptomatic cohorts[20].

Cervical SC magnetic resonance spectroscopy (MRS) in asymptomatic SOD1 carriers reveals a similar neurometabolic profile compared to symptomatic ALS patients, namely reduced NAA/Cr and NAA/Myo ratios[21]. SC pathology in *c9orf72* mutations carriers however has not been studied *in vivo* to date. In recent years, spinal imaging has seen momentous developments, and the combination of novel biomarkers[22] and analysis pipelines[23] allows the quantitative characterisation of disease-associated grey and WM profiles, captures progressive changes and may have a role in diagnostic applications[24,25,26,27].

The primary objective of this study is the comprehensive characterisation of cervical SC GM and WM integrity in asymptomatic *c9orf72* hexanucleotide carriers. The secondary aim is to appraise longitudinal changes and discuss associations with brain pathology.

Methods

This study was approved by the Ethics Committee (Institutional Research Board) of the Pitié-Salpêtrière University Hospital (Paris) and all the participants provided informed consent (PrevDemAls, NCT02590276).

Seventy-two individuals from 46 families were enrolled in this prospective longitudinal study between 2015 and 2017, they were all first-degree relatives of *c9orf72* mutation carriers affected by either ALS or FTD. All the 72 subjects were tested for the *c9orf72* genetic status by repeat-primed-PCR and by Southern blot on lymphocytic DNA. Forty out of 72 participants carried the pathogenic expansion (C9+) with more than 30 GGGGCC repeats. The 32 first-degree relatives not carrying the pathogenic expansion (C9-) were used as a control group to reduce the genetic variability in the general population. All participants underwent a standardised and comprehensive neurological and neuropsychological examination with particular attention to upper and lower motor neuron signs, bulbar dysfunction, cognitive and behavioural changes to ensure that they were asymptomatic for both ALS and FTD[19]. The clinical evaluation was performed by an investigator (ILB) who was blinded to the genetic status of the subjects. The participants were considered asymptomatic if they did not report any neurological symptoms and did not exhibit any neurological signs. Subjects presenting with cramps, fasciculations or fatigue or any other neurological signs were excluded from the study. Other exclusion criteria included: age younger than 18 years, comorbid SC diseases (previous surgery, trauma, severe disk protrusion, or other neurological conditions involving the SC) and any medical condition precluding MRI.

Similarly to previous studies, the expected age of symptom onset in *c9orf72* carriers was estimated by averaging the age of onset of affected individuals in the same family[28]. All subjects underwent a follow-up clinical assessment and imaging 18 months after the initial timepoint.

Cervical spinal cord acquisition protocol

All the participants underwent standardised 3 Tesla cervical SC magnetic resonance (MR) imaging using a Siemens 3T Magnetom Prisma MRI scanner with a 64-channel head and neck coil. A sagittal 3D T2-weighted fast spin-echo SPACE sequence was used for vertebral level identification with slab selective excitation, voxel size = 0.8×0.8×0.8 mm^3, FOV = 256×256 mm^2, 72 sagittal slices, TR/TE = 1500/131 ms, acceleration factor R = 2. High-resolution structural anatomical images between C2 and C7 spinal levels were acquired with an axial 2D T2*-weighted multi-echo gradient echo sequence (MEDIC) to provide high in-plane GM-WM contrast[22]. Voxel size = 0.5×0.5×5 mm^3, FOV = 180×180 mm^2, TR/effective TE = 500/22 ms, flip angle = 30°, seven slices were acquired. The slice orientation and gap were adjusted for each participant so that each slice was acquired at the middle of each cervical vertebral level and the slab was perpendicular to the SC axis.

Spinal diffusion tensor imaging (DTI) data were acquired using a reduced field of view EPI sequence (ZOOMit) with one axial slab covering the C2-C7 vertebral levels. Slices were placed at the middle of each vertebral level and perpendicularly to the SC axis. Imaging parameters were: b = 1000 s/mm2, 60 diffusion-encoding gradient directions, voxel size = 0.8×0.8×5 mm^3, FOV = 45×128 mm^2, TR/TE = 500/58 ms, flip angle = 90°, and cardiac gating was utilised.

Spinal cord MRI data processing

Imaging data were processed using the Spinal Cord Toolbox v3.1 (SCT)[29] by two investigators blinded to the genetic status and clinical profile of the participants (GQ, ML). Images were first visually inspected for incidental findings and underwent quality control. Two baseline C9- scans and three C9+ follow-up scans were excluded due to poor image quality and motion artefacts. The sagittal 3D T2-weighted volume was automatically segmented using PropSeg[30] and non-linearly registered to the MNI-Poly-AMU template[31] to provide automated vertebral labelling for each subject's scan. Subsequently, for each vertebral level, automatic SC segmentation and ROI delimitation were performed based on the axial T2*-weighted images using the probabilistic WM/GM AMU and WM tract atlases, as previously described[32]. GM and WM were automatically segmented on the T2*-weighted data and WM- cross sectional area (CSA) and GM-CSA were estimated in mm^2 [22].

DTI images were co-registered to the MNI-Poly-AMU template[32]. Motion and eddy-current corrections were first performed, and the four main diffusivity metrics were subsequently computed; fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD) and mean

diffusivity (MD). Using template-based analyses, DTI parameters were calculated for the corticospinal tracts (CSTs) and for the dorsal columns bilaterally and averaged. The MRI protocol used in the study and the results of data analysis are presented in **Figure 1**.

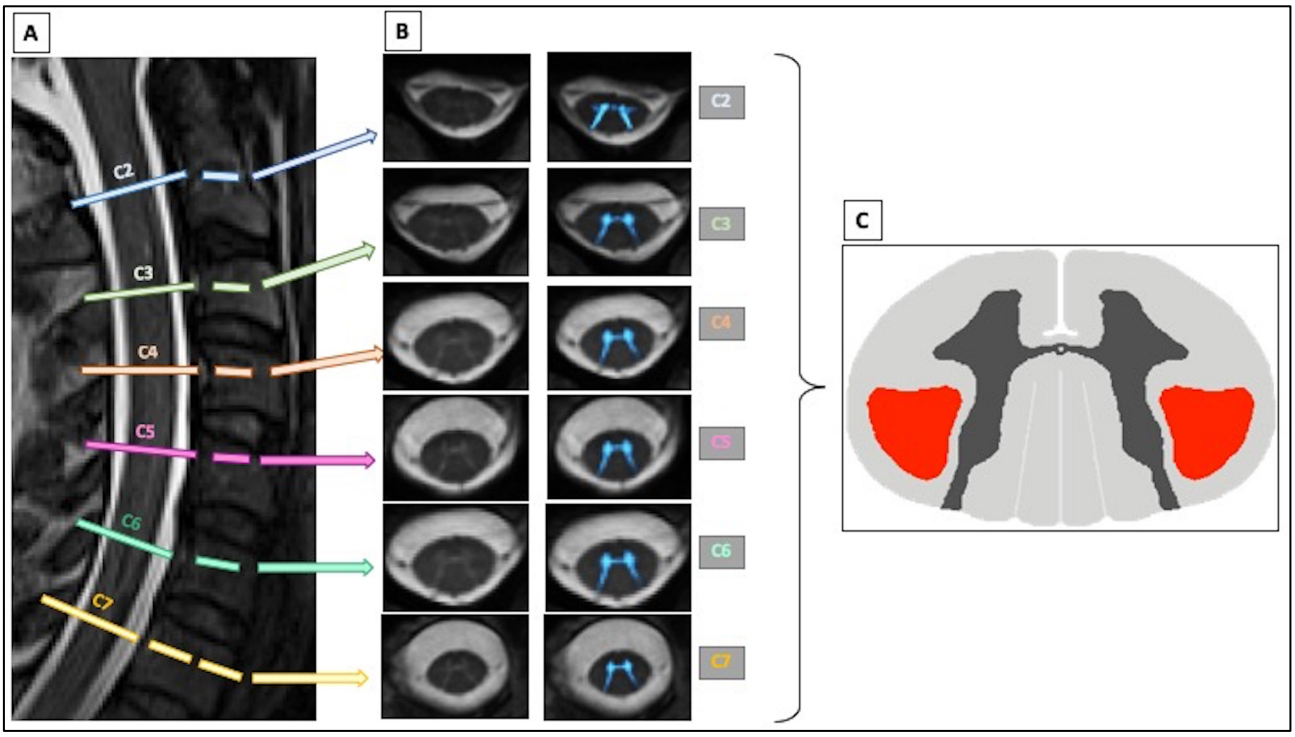

Figure 1. The multiparametric cervical spinal cord MRI protocol here exemplified in a C9-negative subject includes (A) a T2-weighted SPACE sagittal sequence for vertebral levels identification, (B) an axial high-contrast 2D T2*-weighted sequence for accurate white and grey matter segmentation and (C) a 64-direction DTI sequence (a FA map describing CSTs region of interest is showed hier). Template-based analysis allows the computation of DTI parameters in the corticospinal tracts. CST = corticospinal tract.

Statistical analysis

Statistical analyses were performed using JMP®, Version 13 Pro (SAS Institute Inc., Cary, NC). The Shapiro-Wilk test was used to test for normality. Descriptive variables such as mean, standard deviation, percentage and range were used to summarise quantitative measures. Group comparisons of normally distributed variables were performed using Student's t-tests. For non-normally distributed data, the 2-tailed unpaired Mann-Whitney U test was used. The Chi-squared test was used to compare categorical data. Spearman's correlation coefficient was used to evaluate correlations. False Discovery Rate (FDR) was applied to each test to correct for multiple comparisons. The significance level was set at $p < 0.05$.

Structural and microstructural differences between *c9orf72* hexanucleotide carriers and non-carriers were assessed using linear mixed-effects models similarly to our previous paper focusing on the cerebral profile of the same cohort[19]. Age at imaging, gender, and mutation status were used

as fixed effects, while family membership was considered as random effect. Family membership was defined as a nominal covariate constituted by a code identifying the family of origin of each subject. This parameter was implemented as a covariate in the model to reduce the possibility that genetic factors others than *c9orf72* status could influence the results in subjects belonging to the same family.

Statistics were first performed on the entire study population and subsequently in two age-defined sub-groups; those younger and those older than 40 years of age. The age cut-off of 40 years was defined based on the mean age of the study population (42 years +/- 12) and according to our previous paper describing cerebral alterations in the same cohort[19] to help the comparative interpretation of the two studies. An additional analysis was performed in a sub-group of subjects who were related to symptomatic ALS patients as opposed to those only having FTD cases in their family.

A mixed model with two levels of hierarchy was used to analyse longitudinal differences between the baseline and the 18-month evaluation.

Results

The demographic and clinical profile of study participants at baseline is presented in **table 1**.

Total population	C9+ subjects	C9- subjects	p-value
n	40	32	
Age (years)	41.7 ± 11.6 (range 23-76)	45.0 ± 13.1 (range 22-70)	0.23
Sex	17M:23F	16M:16F	0.60
Phenotype in family: FTD/ALS/mixed	15/3/22	14/2/16	0.96
Estimated number of years to onset	-17.95 ± 11.24	N/a	N/a
Expected age of onset	59.1 ± 6.2	N/a	N/a
ALSFRS-r score (/48)	48	48	0.99
MMSE score (/30)	28.8 ± 1.5	28.6 ± 1.3	0.34
FAB score (/18)	16.8 ± 1.4	17.1 ± 0.9	0.39
MDRS score (/144)	141.5 ± 3.2	141.4 ± 2.6	0.54
Height (cm)	169 ± 9 (range 150 – 185)	172 ± 8 (range 160 – 187)	0.13
Subjects < 40 years	**C9+ subjects**	**C9- subjects**	**p-value**
n	16	12	
Age (years)	32.1 ± 4.3	32.0 ± 5.2	0.69
Gender	8M:8F	5M:7F	0.53
Phenotype in family: FTD/ALS/mixed	121/1/3	8/1/3	0.82
Estimated number of years to onset	-24.16 ± 10.51	N/a	N/a
Height (cm)	171 ± 9.26 (range 152 – 185)	169 ± 8.45 (range 160 – 187)	0.65
Subjects > 40 years	**C9+ subjects**	**C9- subjects**	**p-value**
n	24	20	N/a
Age (years)	53.5 ± 9.12	48.9 ± 9.7	0.12
Gender	10M:14F	9M:11F	0.63
Familial phenotype: FTD/ALS/mixed	3/2/19	6/1/13	0.70
Estimated number of years to onset	-10.52 ± 9.31	N/a	N/a
Height (cm)	168 ± 9.64 (range 150 – 183)	173 ± 6.7 (range 162 – 187)	0.06

Table 1: Demographic and clinical characteristics of study participants. Values are expressed as mean ± standard deviation, ratio and values' range. C9+ = carriers of the *c9orf72* mutation. Familial phenotype: FTD: frontotemporal dementia; ALS: amyotrophic lateral sclerosis; FTD/ALS: comorbid amyotrophic lateral sclerosis and frontotemporal dementia. ALSFRS-r: ALS functional rating scale revised. MMSE: Mini Mental State Examination. FAB: Frontal Assessment Battery. MDRS: Matting Dementia Rating Scale. Next to each neuropsychological score is indicated, in brackets, the score obtained if all items of the test are correctly performed.

No significant differences were observed for any of the parameters between the C9+ and C9- cohort.

All the subjects were re-evaluated 18-months later. None of the subjects reported any symptoms or exhibited signs of neurological disease. No significant differences were observed between the main demographic parameters either.

The effect of *c9orf72* status on total cervical SC cross-sectional area (CSA)

No significant differences were detected at baseline when comparing total SC CSA of C9+ and C9- subjects at vertebral levels C2-C7 ($p > 0.05$). Even after splitting the cohorts based on age (40), no CSA reductions were identified in the C9+ group. No significant differences were observed either when considering only participants with a family history of ALS, but a trend for SC atrophy was observed in C9+ subjects. The complete dataset is shown in **supplementary table 1**. Similarly, no total CSA reductions were detected at the second timepoint between C9+ and C9- subjects (**supplementary table 2**) and no progressive cervical SC atrophy was detected between the two timepoints in either group (**supplementary table 3**).

The effect of *c9orf72* status on cervical spinal cord grey matter (GM) cross-sectional area (CSA)

No significant GM CSA differences were detected between the baseline scans of C9+ and C9- subjects at vertebral levels C2-C7 ($p > 0.05$). Even after splitting the cohorts based on age (40), no GM CSA reductions were identified in the C9+ group. The complete dataset is presented in **supplementary table 4**. Similarly, no GM reductions were detected at the second timepoint between C9+ and C9- subjects (**supplementary table 5**) and no progressive GM atrophy was detected between the two timepoints in either group (**supplementary table 6**). No significant differences were observed either when only considering participants with a family history of ALS (**supplementary table 5**).

The effect of *c9orf72* status on cervical spinal cord white matter (WM) cross-sectional area (CSA).

No significant WM CSA reductions were observed in C9+ patients compared to C9- subjects between vertebral levels C2-C7 across the entire study population and in the subgroup of subjects younger than 40 years of age ($p > 0.05$) (**Supplementary table 7**). However, significant WM atrophy was detected at each vertebral level in C9+ subjects older than 40 years of age compared to C9- subjects belonging to the same age group (**table 2**). No correlation was observed between WM CSA and age, between WM CSA and expected time to symptom onset, or the estimated age of symptom onset ($p > 0.05$).

Subjects > 40 years	C9+ subjects	C9- subjects	p-value
WM CSA C2	49.421 ± 24.308	67.694 ± 22.560	**0.020**
WM CSA C2-C3	46.603 ± 20.538	66.413 ± 23.096	**0.010**
WM CSA C3-C4	50.432 ± 24.312	70.193 ± 23.311	**0.012**
WM CSA C4-C5	48.058 ± 22.376	63.900 ± 25.879	**0.032**
WM CSA C5-C6	43.754 ± 21.658	59.868 ± 26.922	**0.027**
WM CSA C6-C7	35.646 ± 22.086	53.413 ± 24.504	**0.037**

Table 2: White matter (WM) cross-sectional area (CSA, mm^2) for C2-C7 vertebral levels presented as mean ± standard deviation in C9+ and C9- subjects older than 40 years of age at the baseline. Using a mixed linear model, significant differences were detected between the two study groups at each cervical vertebral level (results are FDR-corrected and adjusted for age, gender and family membership). C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Similar differences were identified between C9+ and C9- patients over 40 at the second time point, (**supplementary table 8**), but no significant WM CSA reduction was observed between the two assessments ($p > 0.05$, complete data shown **in supplementary table 9**).

No significant differences were observed when considering only C9+ and C9- participants with a family history of ALS (**supplementary table 7, 8 and 9**), even if a clear statistical trend to WM atrophy was observed (FDR-corrected $p = 0.09$ for CSA at C2 level, $p = 0.21$ at C2-C3, $p = 0,12$ at C3-C4, $p = 0.17$ at C4-C5, $p = 0.20$ for C5-C6 and $p = 0.13$ for C6-C7).

The effect of the *c9orf72* status on DTI parameters.

At baseline, no significant differences were identified between C9+ and C9- subjects in the four main DTI parameters (FA, AD, MD, RD) in the CSTs irrespective of studying the entire cohort or splitting them according to age (**supplementary table 10**). At 18-month follow-up, significant FA reduction was observed in the CSTs of the entire C9+ cohort compared to C9- (FDR-corrected p-value 0.029) and also in older C9+ subjects compared to C9- subjects over 40 (FDR-corrected p-value = 0.031) (**table 3**). Other than FA alterations, no differences in AD, MD or RD were detected between the study groups.

Total population	C9+ subjects	C9- subjects	p-value
FA lateral CSTs	0.360 ± 0.1249	0.406 ± 0.1080	**0.029***
AD lateral CSTs	0.0015 ± 0.0005	0.0016 ± 0.0006	0.83
MD lateral CSTs	0.0012 ± 0.0004	0.0011 ± 0.0004	0.89
RD lateral CSTs	0.0110 ± 0.0004	0.0009 ± 0.0004	0.53
Subjects < 40 years	**C9+ subjects**	**C9- subjects**	**p-value**
FA lateral CSTs	0.350 ± 0.1462	0.432 ± 0.1378	0.12
AD lateral CSTs	0.0016 ± 0.0006	0.0014 ± 0.0005	0.39
MD lateral CSTs	0.0012 ± 0.0005	0.0010 ± 0.0003	0.30
RD lateral CSTs	0.0011 ± 0.0005	0.0008 ± 0.0002	0.16
Subjects > 40 years	**C9+ subjects**	**C9- subjects**	**p-value**
FA lateral CSTs	0.368 ± 0.1030	0.387 ± 0.0676	**0.031***
AD lateral CSTs	0.0015 ± 0.0004	0.0016 ±- 0.0006	0.70
MD lateral CSTs	0.0011 ± 0.0003	0.0017 ± 0.0004	0.71
RD lateral CSTs	0.0009 ± 0.0004	0.0010 ± 0.0004	0.90
Subjects with a family history of ALS	**C9+**	**C9-**	**p-value**
FA lateral CSTs	0.392 +/- 0.024	0.416 +/- 0.029	0.64
AD lateral CSTs	0.0016 +/- 0.0001	0.0016 +/- 0.0001	0.60
MD lateral CSTs	0.0087 +/- 0.0058	0.0012 +/- 0.006	0.36
RD lateral CSTs	0.0010 +/- 0.00008	0.0010 +/- 0.0001	0.45

Table 3. DTI parameters in the corticospinal tracts expressed as mean ± standard deviation in the entire population and in age-defined subgroups (younger and older than 40 years of age) at the second timepoint. Using mixed linear models, a significant FA reduction was observed in the total population and in subjects older than 40 years of age adjusting for age, gender and height, while no significant difference was present in subjects younger than 40 years of age. *statistically significant differences.

Significant longitudinal CST FA reduction was only observed in subjects older than 40 years (p-value = 0.033). No other progressive diffusivity changes were detected over time (**supplementary table 11**).

The same DTI analyses were also performed in the dorsal columns. No significant differences were detected between C9+ and C9- subjects for any of the DTI parameters ($p > 0.05$) neither in the entire study population, nor after stratifying by age (< 40 and > 40 years of age) (**Supplementary table 12 and 13**).

The effect of family history (ALS versus FTD) on diffusivity parameters

Intriguingly, asymptomatic C9+ subjects with a family history of ALS (n=24) exhibited significant CSTs FA reduction at baseline compared to C9- subjects. (0.61 +/- 0.046 in C9- subjects versus 0.38 +/- 0.038 in C9+ subjects, p = 0.0034). These observations were only evident when considering subjects older than 40 years of age. The results were further confirmed by the follow-up data. Furthermore, no significant differences were observed in dorsal column DTI parameters between C9+ and C9- subjects with a family history of ALS ($p > 0.05$), suggesting that selective CSTs degeneration is an early event and confirming that in ALS the dorsal columns remain relatively intact in the presymptomatic and early stages of the disease.

Discussion

This study characterises cervical spinal cord WM and GM changes in a large cohort of asymptomatic *c9orf72* mutation carriers and evaluates the findings based on mutation status, and family history of ALS versus FTD. Despite considerable advances in spinal imaging technology, no study has been dedicated to date to the characterisation of *c9orf72*-associated SC pathology.

The main finding of our study is that C9+ subjects older than 40 years of age exhibit considerable WM atrophy at each vertebral level in conjunction with progressive pyramidal tract FA reductions. The lack of apparent GM involvement and detectable WM pathology in subjects younger than 40, are other important findings.

Early detection of WM alterations and ensuing GM degeneration has been reported in both genetically determined FTD[28] and ALS[12]. The effect of age on presymptomatic spinal metrics identified in this study was not detected at the cerebral level in the same subjects[19]. On the contrary, structural and microstructural brain changes could be readily detected in C9+ subjects younger than 40. Considerable cerebral changes in young C9+ individuals have also been reported in other studies[14,15,17,33]. The divergent chronological patterns between longitudinal cerebral and spinal alterations offer novel insights about the propagation of *c9orf72*-associated conditions. Cerebral changes in young asymptomatic C9+ subjects have been previously interpreted as neurodevelopmental or early degenerative processes[16]. The link between age and accruing SC pathology detected in our study suggests incremental neurodegeneration.

No CST FA reductions were detected at baseline, but these was evident in subjects older than 40 years of age on 18-month follow-up, suggesting that incremental degeneration may take place when mutation carriers are approaching symptom onset. These conclusions remain speculative since the expected time of onset was estimated based on symptomatic family members and considerable intra-familial heterogeneity exists in symptom onset[34]. Further follow-up at a later time point is required to reassuringly test these hypotheses.

Another integrative interpretation of our findings is that *c9orf72*-associated changes are primarily cerebral initially, confined to brain regions at a younger age, and show progressive propagation to spinal regions. This would support emerging concepts of corticofugal pathological spread in ALS and FTD[35]. The precise molecular mechanisms underlying *c9orf72*-associated degeneration remains largely unknown, even if putative cell-to-cell propagation of the DPRs and TDP-43 inclusions has been proposed[36]. Despite the detection sensitivity of novel spinal imaging

techniques in ALS[24,25,26,37,38,39], the lack of imaging findings in younger mutation carriers may be due to technological limitations and does not necessarily represent lack of pathology.

Presymptomatic imaging in *c9orf72* has a dual academic and clinical relevance. Hexanucleotide repeats may eventually manifest in a primarily cerebral condition (FTD) or in the multisystem cerebro-spinal phenotype ALS or ALS-FTD. No accurate indicators currently exist to predict future conversion; therefore, the assessment of spinal metrics may be the key to appraise the risk of phenotypic conversion to ALS versus FTD. In our cohort, FA reduction was observed at baseline when analysis was restricted to subjects related to symptomatic ALS patients. Additionally, an important trend for total CSA reduction was observed in subjects with a family history of ALS. No systematic studies are currently available regarding the relative risk of developing ALS versus FTD depending on the phenotypic manifestation of the mutation in a given family. Existing studies suggest that the imaging profile of *c9orf72* ALS and FTD patients is different, with ALS patients presenting with more prominent motor system involvement compared to FTD patients[40]. The presymptomatic corticospinal tract FA reduction detected in our study could indicate a higher risk of conversion to ALS than FTD. The results are further supported by the lack of dorsal column diffusivity changes, suggesting that selective FA reduction in the CSTs could be an early event in the natural history of the disease in subjects destined to develop ALS. Robust, prospective longitudinal studies are needed to track young (<40) patients with multimodal spinal and cerebral imaging – preferably with a panel of wet biomarkers and neurophysiology – until symptomatic conversion to ascertain which early markers best predict conversion to ALS and which may be indicative of conversion risk to FTD. SC imaging metrics may be particularly well suited to identify those at risk of converting to ALS as opposed to relying on cerebral imaging indices alone. An obvious advantage of spinal imaging, compared to cerebral imaging, is that it has the potential to appraise both the lower (spinal-GM) and upper (CST DTI, WM-CSA) motor neuron components of the motor system separately.

In this study, SC WM atrophy was observed at baseline without concomitant diffusivity alterations. This stands in contrast with the widespread DTI alterations at the cerebral level in the same cohort of patients[19] as well as previous cerebral *c9orf72* studies in asymptomatic or symptomatic patients[14, 15,40,41]. This discrepancy could be due to the divergent sensitivity profile of spinal and cerebral DTI. Advanced DTI methods are particularly challenging at the spinal level because of the small cross-sectional area of the human cord, physiological motions due to respiration, vascular pulsation effects, partial volume effects due to the surrounding cerebrospinal

fluid and magnetic field inhomogeneities around the intervertebral discs and the lungs[42]. It is also important to acknowledge that DTI metrics such as FA or MD are not pathology-specific but composite measures of WM integrity[43]. RD and AD are thought to be histologically more accurate representing myelin and axonal changes with more specificity. Furthermore, emerging diffusion imaging techniques such as Neurite orientation dispersion and density imaging (NODDI) may have superior detection sensitivity and be particularly useful in detecting crossing fibre degeneration[44,45]. NODDI is an advanced diffusion imaging technique that has already been applied to highlight widespread presymptomatic changes in asymptomatic C9+ subjects from the same cohort[46]. Accordingly, WM atrophy, which is closely related to neurite density and is readily measurable in the cervical SC, may be a more sensitive marker of pathology than DTI metrics at the early asymptomatic stage of the disease. Spinal DTI changes are more likely to be detected when multiple cellular events have taken place, such as axonal degeneration, demyelination, membrane disintegration and fibre orientation loss. A few spinal cord imaging studies have validated the histological and microstructural correlates of diffusivity metrics, but the pathological underpinnings of WM volumes are especially poorly characterised[47]. Our current understanding of the microstructural correlates of MRI metrics in neurologic conditions are largely putative, further animal studies are needed, and robust human combined post mortem MRI and histology studies are required[48,49].

Our study is not without limitations. Even if our entire study population is relatively large considering the rarity of the *c9orf72* presymptomatic carriers, the number of subjects in each group after the sub-classification leads to limited sample sizes and the statistical power of the model is therefore reduced.

Notwithstanding these limitations, our study confirms that presymptomatic imaging is a powerful tool to investigate the temporal and spatial propagation of pathology and may have an important prognostic role. The establishment of the sensitivity profile of multiple imaging metrics in the asymptomatic phase of the disease has implications for prognostication, genetic counselling and future therapeutic trials. Combining these imaging measures with wet biomarkers such as neurofilament light chains[50] may increase the diagnostic, prognostic and monitoring potential of these indices further. Ultimately, the careful synthesis of cerebral and spinal imaging, the integration of biofluid markers, robust multi-timepoint longitudinal designs and post mortem validation will be needed to map the sequential spread of *c9orf72*-associated pathology.

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Author contributions

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Potential conflict of interest

The authors have no competing financial interests to declare in relation this paper.

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Supplementary table 1. Total cross-sectional area (CSA, mm^2) for vertebral levels C2-C7 presented as mean and standard deviation in C9+ and C9- subjects at baseline. Comparisons are FDR corrected and adjusted for age, gender and family membership.
C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**C9+**	**C9-**	**p-value**
CSA C2	79.306 +/- 1.126	79.568 +/- 1.294	0.89
CSA C2-C3	79.830 +/- 1.321	79.484 +/- 1.492	0.84
CSA C3-C4	82.123 +/- 1.456	81.767 +/- 1.622	0.77
CSA C4-C5	79.895 +/- 1.798	79.609 +/- 2.031	0.75
CSA C5-C6	73.214 +/- 1.743	72.622 +/- 1.969	0.76
CSA C6-C7	62.386 +/- 1.852	61.957 +/- 2.091	0.89
Subjects < 40 years	**C9+**	**C9-**	**p-value**
CSA C2	77.651 +/- 1.632	77.743 +/- 1.811	0.64
CSA C2-C3	79.100 +/- 2.062	78.663 +/- 2.287	0.82
CSA C3-C4	80.621 +/- 2.519	80.205 +/- 2.706	0.96
CSA C4-C5	79.208 +/- 3.203	77.676 +/- 3.699	0.99
CSA C5-C6	73.058 +/- 2.798	71.501 +/- 3.231	0.93
CSA C6-C7	62.422 +/- 2.758	60.356 +/- 3.059	0.74
Subjects > 40 years	**C9+**	**C9-**	**p-value**
CSA C2	79.305 +/- 1.475	79.880 +/- 1.623	0.89
CSA C2-C3	79.198 +/- 1.709	79.523 +/- 1.880	0.88
CSA C3-C4	81.409 +/- 1.790	81.344 +/- 1.970	0.87
CSA C4-C5	78.265 +/- 2.185	79.006 +/- 2.404	0.78
CSA C5-C6	71.050 +/- 2.263	72.143 +/- 2.490	0.81
CSA C6-C7	60.277 +/- 2.477	61.429 +/- 2.726	0.80
Subjects with a family history of ALS	**C9+**	**C9-**	**p-value**
CSA C2	79.147 +/- 1.393	80.993 +/- 1.674	0.54
CSA C2-C3	79.951 +/- 1.577	81.572 +/- 1.896	0.86
CSA C3-C4	82.351 +/- 1.754	84.584 +/- 2.108	0.68
CSA C4-C5	79.337 +/- 2.176	82.618 +/- 2.616	0.73
CSA C5-C6	73.055 +/- 2.174	74.839 +/- 2.613	0.98
CSA C6-C7	62.601 +/- 2.481	64.214 +/- 2.982	0.93

Supplementary table 2. Total cross-sectional area (CSA, mm^2) for vertebral levels C2-C7 presented as mean and standard deviation in C9+ and C9- subjects at 18-month follow-up. Comparisons are FDR corrected and adjusted for age, gender and family membership.
C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**C9+**	**C9-**	**p-value**
CSA C2	79.663 +/- 1.004	78.523 +/- 1.139	0.54
CSA C2-C3	80.066 +/- 1.116	78.905 +/- 1.266	0.31
CSA C3-C4	84.968 +/- 1.252	82.021 +/- 1.419	0.48
CSA C4-C5	81.064 +/- 2.246	80.276 +/- 2.593	0.72
CSA C5-C6	76.201 +/- 1.534	74.280 +/- 1.840	0.55
CSA C6-C7	63.826 +/- 1.558	63.518 +/- 1.843	0.75
Subjects < 40 years	**C9+**	**C9-**	**p-value**
CSA C2	77.651 +/- 1.632	77.743 +/- 1.811	0.37
CSA C2-C3	79.100 +/- 2.062	78.663 +/- 2.287	0.63
CSA C3-C4	80.621 +/- 2.519	80.205 +/- 2.706	0.36
CSA C4-C5	79.208 +/- 3.203	77.676 +/- 3.699	0.97
CSA C5-C6	73.058 +/- 2.798	71.501 +/- 3.231	0.22
CSA C6-C7	62.422 +/- 2,758	60.356 +/- 3.059	0.43
Subjects > 40 years	**C9+**	**C9-**	**p-value**
CSA C2	80.020 +/- 1.465	79.821 +/- 1.691	0.86
CSA C2-C3	79.844 +/- 1.481	79.070 +/- 1.710	0.61
CSA C3-C4	84.083 +/- 1.578	82.695 +/- 1.822	0.37
CSA C4-C5	82.163 +/- 1.785	81.110 +/- 2.062	0.29
CSA C5-C6	74.662 +/- 1.953	74.483 +/- 2.255	0.75
CSA C6-C7	62.236 +/- 1.935	64.085 +/- 2.234	0.58
Subjects with a family history of ALS	**C9+**	**C9-**	**p-value**
CSA C2	79.584 +/- 1.350	80.703 +/- 1.546	0.93
CSA C2-C3	79.718 +/- 1.382	81.554 +/- 1.583	0.80
CSA C3-C4	84.677 +/- 1.322	85.912 +/- 1.515	0.79
CSA C4-C5	79.885 +/- 3.308	84.869 +/- 3.915	0.28
CSA C5-C6	76.160 +/- 1.752	77.723 +/- 2.145	0.56
CSA C6-C7	63.662 +/- 1.816	66.612 +/- 2.224	0.74

Supplementary table 3. Total SC cross-sectional area (CSA, mm^2) for vertebral levels C2-C7 presented as mean and standard deviation in C9+ subjects at baseline and at 18-month follow-up. Comparisons are FDR corrected and adjusted for age, gender and family membership.
C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**Baseline**	**18-months evaluation**	**p-value**
CSA C2	80.165 +/- 1.210	79.584 +/- 1.266	0.60
CSA C2-C3	80.974 +/- 1.386	79.718 +/- 1.451	0.73
CSA C3-C4	83.969 +/- 1.551	84.677 +/- 1.624	0.66
CSA C4-C5	81.497 +/- 3.065	79.885 +/- 3.208	0.78
CSA C5-C6	74.226 +/- 1.708	76.160 +/- 1.788	0.22
CSA C6-C7	63.053 +/- 2.025	63.662 +/- 2.119	0.71
Subjects <40 years	**Baseline**	**18-months evaluation**	**p-value**
CSA C2	77.670 +/- 1.773	78.751 +/- 1.763	0.42
CSA C2-C3	78.839 +/- 2.943	79.366 +/- 1.941	0.49
CSA C3-C4	80.541 +/- 2.745	84.351 +/- 2.400	0.48
CSA C4-C5	79.090 +/- 4.618	77.482 +/- 3.345	0.91
CSA C5-C6	72.892 +/- 2.404	75.472 +/- 2.737	0.36
CSA C6-C7	62.086 +/- 2.473	62.909 +/- 2.554	0.78
Subjects > 40 years	**Baseline**	**18-months evaluation**	**p-value**
CSA C2	79.836 +/- 1.361	79.676 +/- 1.224	0.75
CSA C2-C3	79.020 +/- 1.585	79.398 +/- 1.426	0.79
CSA C3-C4	80.959 +/- 1.797	83.223 +/- 1.617	0.22
CSA C4-C5	80.858 +/- 1.792	77.948 +/- 1.991	0.11
CSA C5-C6	73.643 +/- 1.745	70.640 +/- 1.939	0.20
CSA C6-C7	61.662 +/- 1.468	58.174 +/- 1.632	0.25
Subjects with a family history of ALS	**Baseline**	**18-months evaluation**	**p-value**
CSA C2	80.165 +/- 1.210	79.584 +/- 1.266	0.73
CSA C2-C3	80.974 +/- 1.386	79.718 +/- 1.451	0.72
CSA C3-C4	83.969 +/- 1.551	84.677 +/- 1.624	0.40
CSA C4-C5	81.497 +/- 3.065	79.885 +/- 3.208	0.78
CSA C5-C6	74.226 +/- 1.708	76.160 +/- 1.788	0.32
CSA C6-C7	63.053 +/- 2.025	63.662 +/- 2.119	0.71

Supplementary table 4. Grey matter (GM) cross-sectional area (CSA, mm^2) at vertebral levels C2-C7 presented as mean and standard deviation in C9+ and C9- subjects at baseline. Comparisons are FDR corrected and adjusted for age, gender and family membership.
C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**C9+ subjects**	**C9- subjects**	**p-value**
GM CSA C2	14.738 +/- 2.529	13.723 +/- 2.446	0.34
GM CSA C2-C3	13.931 +/- 1.892	13.578 +/- 2.572	0.31
GM CSA C3-C4	15.050 +/- 2.190	14.350 +/- 2.506	0.32
GM CSA C4-C5	14.414 +/- 2.408	13.368 +/- 3.178	0.30
GM CSA C5-C6	13.372 +/- 2.132	13.047 +/- 2.793	0.38
GM CSA C6-C7	11.169 +/- 2.300	11.097 +/- 2.640	0.49
Subjects < 40 years	**C9+ subjects**	**C9- subjects**	**p-value**
GM CSA C2	13.388 +/- 2.256	14.102 +/- 1.791	0.75
GM CSA C2-C3	13.357 +/- 2.366	13.585 +/- 2.114	0.73
GM CSA C3-C4	13.954 +/- 2.745	14.493 +/- 1.850	0.72
GM CSA C4-C5	13.171 +/- 3.402	13.236 +/- 2.462	0.73
GM CSA C5-C6	12.731 +/- 3.848	12.786 +/- 2.033	0.75
GM CSA C6-C7	10.679 +/- 3.094	10.541 +/- 2.339	0.43
Subjects > 40 years	**C9+ subjects**	**C9- subjects**	**p-value**
GM CSA C2	15.173 +/- 2.822	14.113 +/- 1.949	0.57
GM CSA C2-C3	14.234 +/- 1.750	13.806 +/- 2.435	0.82
GM CSA C3-C4	15.395 +/- 2.408	14.433 +/- 2.938	0.31
GM CSA C4-C5	14.669 +/- 2.403	13.714 +/- 2.557	0.66
GM CSA C5-C6	13.774 +/- 2.182	12.7766 +/- 2.765	0.38
GM CSA C6-C7	11.645 +/- 2.235	11.384 +/- 2.342	0.56
Subjects with a family history of ALS	**C9+**	**C9-**	**p-value**
GM CSA C2	15.051 +/- 0.429	14.352 +/- 0.505	0.27
GM CSA C2-C3	14.319 +/- 0.330	14.543 +/- 0.389	0.86
GM CSA C3-C4	15.213 +/- 0.474	15.121 +/- 0.558	0.55
GM CSA C4-C5	14.871 +/- 0.458	14.279 +/- 0.540	0.30
GM CSA C5-C6	13.568 +/- 0.454	13.760 +/- 0.536	0.86
GM CSA C6-C7	11.774 +/- 0.510	11.571 +/- 0.580	0.68

Supplementary table 5. Grey matter (GM) cross-sectional area (CSA, mm^2) at vertebral levels C2-C7 presented as mean and standard deviation in C9+ and C9- subjects at 18-month follow-up. Comparisons are FDR corrected and adjusted for age, gender and family membership.
C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**C9+ subjects**	**C9- subjects**	**p-value**
GM CSA C2	14.220 +/- 3.018	13.418 +/- 0.351	0.65
GM CSA C2-C3	14.509 +/- 3.850	13.503 +/- 2.001	0.83
GM CSA C3-C4	14.803 +/- 2.812	14.405 +/- 1.772	0.36
GM CSA C4-C5	14.390 +/- 3.050	13.530 +/- 2.492	0.65
GM CSA C5-C6	13.485 +/- 2.346	12.566 +/- 1.988	0.44
GM CSA C6-C7	11.798 +/- 2.566	10.903 +/- 1.751	0.27
Subjects < 40 years	**C9+ subjects**	**C9- subjects**	**p-value**
GM CSA C2	14.402 +/- 1.565	13.927 +/- 1.892	0.46
GM CSA C2-C3	14.509 +/- 3.850	13.653 +/- 1.940	0.67
GM CSA C3-C4	15.710 +/- 3.146	14.802 +/- 1.471	0.69
GM CSA C4-C5	14.710 +/- 3.345	14.280 +/- 1.969	0.72
GM CSA C5-C6	13.184 +/- 2.707	13.232 +/- 1.912	0.36
GM CSA C6-C7	11.037 +/- 2.669	10.580 +/- 1.235	0.41
Subjects > 40 years	**C9+ subjects**	**C9- subjects**	**p-value**
GM CSA C2	14.037 +/- 2.166	13.428 +/- 2.252	0.31
GM CSA C2-C3	14.098 +/- 2.000	13.799 +/- 2.358	0.94
GM CSA C3-C4	14.156 +/- 2.188	14.407 +/- 2.121	0.72
GM CSA C4-C5	14.198 +/- 2.815	13.559 +/- 1.242	0.50
GM CSA C5-C6	13.804 +/- 1.914	13.072 +/- 1.888	0.87
GM CSA C6-C7	12.618 +/- 0.560	11.302 +/- 2.099	0.19
Subjects with a family history of ALS	**C9+**	**C9-**	**p-value**
GM CSA C2	15.051 +/- 0.429	14.352 +/- 0.505	0.27
GM CSA C2-C3	14.319 +/- 0.330	14.543 +/- 0.389	0.86
GM CSA C3-C4	15.213 +/- 0.474	15.121 +/- 0.558	0.55
GM CSA C4-C5	14.871 +/- 0.458	14.279 +/- 0.540	0.30
GM CSA C5-C6	13.568 +/- 0.454	13.760 +/- 0.536	0.86
GM CSA C6-C7	11.774 +/- 0.510	11.571 +/- 0.580	0.68

Supplementary table 6. Grey matter (GM) cross-sectional area (CSA, mm^2) at vertebral levels C2-C7 presented as mean and standard deviation in C9+ subjects at baseline and at 18-month follow-up. P-values are FDR corrected and adjusted for age, gender and family membership.
C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**Baseline**	**18-months evaluation**	**p-value**
GM CSA C2	14.738 +/- 2.529	14.220 +/- 3.018	0.16
GM CSA C2-C3	13.931 +/- 1.892	14.509 +/- 3.850	0.69
GM CSA C3-C4	15.050 +/- 2.190	14.803 +/- 2.812	0.55
GM CSA C4-C5	14.414 +/- 2.408	14.390 +/- 3.050	0.82
GM CSA C5-C6	13.372 +/- 2.132	13.485 +/- 2.346	0.90
GM CSA C6-C7	11.169 +/- 2.300	11.798 +/- 2.566	0.40
Subjects <40 years	**Baseline**	**18-months evaluation**	**p-value**
GM CSA C2	13.388 +/- 2.256	14.402 +/- 1.565	0.45
GM CSA C2-C3	13.357 +/- 2.366	14.509 +/- 3.850	0.36
GM CSA C3-C4	13.954 +/- 2.745	15.710 +/- 3.146	0.16
GM CSA C4-C5	13.171 +/- 3.402	14.710 +/- 3.345	0.94
GM CSA C5-C6	12.731 +/- 3.848	13.184 +/- 2.707	0.89
GM CSA C6-C7	10.679 +/- 3.094	11.037 +/- 2.669	0.73
Subjects > 40 years	**Baseline**	**18-months evaluation**	**p-value**
GM CSA C2	15.173 +/- 2.822	14.037 +/- 2.166	0.24
GM CSA C2-C3	14.234 +/- 1.750	14.098 +/- 2.000	0.65
GM CSA C3-C4	15.395 +/- 2.408	14.156 +/- 2.188	0.74
GM CSA C4-C5	14.669 +/- 2.403	14.198 +/- 2.815	0.72
GM CSA C5-C6	13.774 +/- 2.182	13.804 +/- 1.914	0.81
GM CSA C6-C7	11.645 +/- 2.235	12.618 +/- 0.560	0.59
Subjects with a family history of ALS	**Baseline**	**18-months evaluation**	**p-value**
GM CSA C2	15.5033 +/- 0.409	14.624 +/- 0.429	0.32
GM CSA C2-C3	14.469 +/- 0.489	15.012 +/- 0.513	0.40
GM CSA C3-C4	15.319 +/- 0.589	15.304 +/- 0.618	0.95
GM CSA C4-C5	14.479 +/- 0.579	14.378 +/- 0.607	0.50
GM CSA C5-C6	13.876 +/- 0.460	13.760 +/- 0.536	0.94
GM CSA C6-C7	11.864 +/- 0.489	12.212 +/- 0.480	0.82

Supplementary table 7. White matter (WM) cross-sectional area (CSA, mm^2) at vertebral levels C2-C7 presented as mean value and standard deviation in C9+ and C9- subjects' populations at the baseline visit. P-values are FDR corrected and adjusted for age, gender and family membership). C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**C9+**	**C9-**	**p-value**
WM CSA C2	60.550 +/- 30.452	67.818 +/- 28.201	0.39
WM CSA C2-C3	56.239 +/- 27.121	66.734 +/-26.171	0.35
WM CSA C3-C4	63.261 +/- 32.576	70.232 +/- 25.912	0.36
WM CSA C4-C5	60.259 +/- 29.394	65.401 +/- 27.304	0.34
WM CSA C5-C6	57.386 +/- 30.116	61.443 +/- 28.987	0.39
WM CSA C6-C7	49.543 +/- 30.295	55.870 +/- 26.266	0.18
Subjects < 40 years	**C9+**	**C9-**	**p-value**
WM CSA C2	66.026 +/- 35.448	75.779 +/- 32.681	0.27
WM CSA C2-C3	65.384 +/- 30.304	69.889 +/- 30.400	0.70
WM CSA C3-C4	68.455 +/- 28.814	80.527 +/- 36.815	0.26
WM CSA C4-C5	68.825 +/- 29.568	77.151 +/- 31.276	0.28
WM CSA C5-C6	62.025 +/- 31.387	74.596 +/- 30.707	0.22
WM CSA C6-C7	57.662 +/- 29.963	64.868 +/- 31.677	0.25
Subjects with a family history of ALS	**C9+**	**C9-**	**p-value**
WM CSA C2	60.378 +/- 6.049	70.078 +/- 6.985	0.09
WM CSA C2-C3	59.638 +/- 5.874	69.458 +/- 6.782	0.21
WM CSA C3-C4	62.101 +/- 6.127	72.203 +/- 7.075	0.12
WM CSA C4-C5	60.513 +/- 6.198	67.281 +/- 7.156	0.17
WM CSA C5-C6	57.901 +/- 6.380	65. 141+/- 7.367	0.20
WM CSA C6-C7	51.129 +/- 6.664	59.095 +/- 7.407	0.13

Supplementary table 8. White matter (WM) cross-sectional area (CSA, mm^2) at vertebral levels C2-C7 presented as mean and standard deviation in C9+ and C9- subjects at 18-month follow-up. Comparisons are FDR corrected and adjusted for age, gender and family membership). C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**C9+**	**C9-**	**p-value**
WM CSA C2	60.305 +/- 26.375	68.298 +/- 25.533	0.34
WM CSA C2-C3	58.798 +/- 23.719	70.069 +/- 21.719	0.41
WM CSA C3-C4	63.527 +/- 26.224	72.049 +/- 24.780	0.47
WM CSA C4-C5	58.847 +/- 27.682	68.179 +/- 27.114	0.56
WM CSA C5-C6	59.597 +/- 27.352	65.270 +/- 27.352	0.48
WM CSA C6-C7	53.722 +/- 26.776	58.400 +/- 24.432	0.53
Subjects < 40 years	**C9+**	**C9-**	**p-value**
WM CSA C2	62.767 +/- 26.684	55.981 +/- 24.191	0.84
WM CSA C2-C3	58.624 +/- 26.379	56.254 +/- 23.627	0.98
WM CSA C3-C4	63.814 +/- 27.044	61.447 +/- 25.949	0.38
WM CSA C4-C5	57.470 +/- 29.390	57.751 +/- 26.247	0.25
WM CSA C5-C6	59.304 +/- 27.145	54.630 +/- 24.285	0.84
WM CSA C6-C7	52.086 +/- 26.052	43.630 +/- 22.762	0.48
Subjects > 40 years	**C9+ subjects**	**C9- subjects**	**p-value**
WM CSA C2	58.132 +/- 26.722	84.416 +/- 31.158	**0.053**
WM CSA C2-C3	58.973 +/- 25.377	85.949+/- 21.739	**0.018**
WM CSA C3-C4	63.240 +/- 26.263	84.259 +/- 23.461	**0.042**
WM CSA C4-C5	61.926 +/- 26.890	79.589 +/- 26.316	**0.049**
WM CSA C5-C6	59.891 +/- 28.445	78.154 +/- 26.678	**0.029**
WM CSA C6-C7	55.256 +/- 28.201	71.903 +/- 21.210	**0.027**
Subjects with a family history of ALS	**C9+**	**C9-**	**p-value**
WM CSA C2	15.051 +/- 0.429	14.352 +/- 0.505	0.27
WM CSA C2-C3	14.319 +/- 0.330	14.543 +/- 0.389	0.86
WM CSA C3-C4	15.213 +/- 0.474	15.121 +/- 0.558	0.55
WM CSA C4-C5	14.871 +/- 0.458	14.279 +/- 0.540	0.30
WM CSA C5-C6	13.568 +/- 0.454	13.760 +/- 0.536	0.86
WM CSA C6-C7	11.774 +/- 0.510	11.571 +/- 0.580	0.68

Supplementary table 9. White matter (WM) cross-sectional area (CSA, mm^2) at vertebral levels C2-C7 presented as mean and standard deviation in C9+ subjects at baseline and at 18-month follow-up. Comparisons are FDR corrected and adjusted for age, gender and family membership). C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**Baseline**	**18-months evaluation**	**p-value**
WM CSA C2	60.550 +/- 30.452	60.305 +/- 26.375	0.87
WM CSA C2-C3	56.239 +/- 27.121	58.798 +/- 23.719	0.55
WM CSA C3-C4	63.261 +/- 32.576	63.527 +/- 26.224	0.92
WM CSA C4-C5	60.259 +/- 29.394	58.847 +/- 27.682	0.98
WM CSA C5-C6	57.386 +/- 30.116	59.597 +/- 27.352	0.71
WM CSA C6-C7	49.543 +/- 30.295	53.722 +/- 26.776	0.57
Subjects < 40 years	**Baseline**	**18-months evaluation**	**p-value**
WM CSA C2	66.026 +/- 35.448	62.767 +/- 26.684	0.32
WM CSA C2-C3	65.384 +/- 30.304	58.624 +/- 26.379	0.28
WM CSA C3-C4	68.455 +/- 28.814	63.814 +/- 27.044	0.26
WM CSA C4-C5	68.825 +/- 29.568	57.470 +/- 29.390	0.13
WM CSA C5-C6	62.025 +/- 31.387	59.304 +/- 27.145	0.27
WM CSA C6-C7	57.662 +/- 29.963	52.086 +/- 26.052	0.36
Subjects > 40 years	**Baseline**	**18-months evaluation**	**p-value**
WM CSA C2	49.421 +/- 24.308	58.132 +/- 26.722	0.69
WM CSA C2-C3	46.603 +/- 20.538	58.973 +/- 25.377	0.65
WM CSA C3-C4	50.432 +/- 24.312	63.240 +/- 26.263	0.68
WM CSA C4-C5	48.058 +/- 22.376	61.926 +/- 26.890	0.42
WM CSA C5-C6	43.754+/- 21.658	59.891 +/- 28.445	0.53
WM CSA C6-C7	35.646 +/- 22.086	55.256 +/- 28.201	0.52
Subjects with a family history of ALS	**Baseline**	**18-months evaluation**	**p-value**
WM CSA C2	64.829 +/- 5.732	59.739 +/- 6.168	0.55
WM CSA C2-C3	63.525 +/- 6.041	58.785 +/- 5.614	0.58
WM CSA C3-C4	69.163 +/- 6.232	61.395 +/- 5.791	0.40
WM CSA C4-C5	66.649 +/- 6.130	59.148 +/- 5.696	0.40
WM CSA C5-C6	66.224 +/- 6.148	56.465 +/- 5.713	0.29
WM CSA C6-C7	58.506 +/- 6.760	49.819 +/- 6.580	0.21

Supplementary table 10. DTI parameters in the corticospinal tracts (CSTs) expressed as mean and standard deviation in the entire population and in age-defined subgroups (younger and older than 40 years of age) at baseline. Comparisons are FDR corrected and adjusted for age, gender and family membership. CSTs: cortico-spinal tracts.
C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**C9+**	**C9-**	**p-value**
FA lateral CSTs	0.396 +/- 0.1278	0.379 +/- 0.1203	0.57
AD lateral CSTs	0.0016 +/- 0.0006	0.0015 +/- 0.0004	0.43
MD lateral CSTs	0.0006 +/- 0.0313	0.0011 +/- 0.0003	0.29
RD lateral CSTs	0.0010 +/- 0.0004	0.0009 +/- 0.0003	0.57
Subjects < 40 years	**C9+**	**C9-**	**p-value**
FA lateral CSTs	0.389 +/- 0.1532	0.346 +/- 0.0894	0.81
AD lateral CSTs	0.0016 +/- 0.0007	0.0015 +/- 0.0005	0.80
MD lateral CSTs	0.0011 +/- 0.0004	0.0012 +/- 0.0003	0.73
RD lateral CSTs	0.0010 +/- 0.0004	0.0009 +/- 0.0004	0.82
Subjects > 40 years	**C9+ subjects**	**C9- subjects**	**p-value**
FA lateral CSTs	0.406 +/- 0.1090	0.402 +/- 0.1361	0.12
AD lateral CSTs	0.0017 +/- 0.0005	0.0015 +/- 0.0005	0.24
MD lateral CSTs	0.0098 +/- 0.0408	0.0011 +/- 0.0003	0.26
RD lateral CSTs	0.0011 +/- 0.0004	0.0009 +/- 0.0004	0.38
Subjects with a family history of ALS	**C9+**	**C9-**	**p-value**
FA lateral CSTs	0.392 +/- 0.024	0.416 +/- 0.029	0.64
AD lateral CSTs	0.0016 +/- 0.0001	0.0016 +/- 0.0001	0.60
MD lateral CSTs	0.0087 +/- 0.0058	0.0012 +/- 0.006	0.36
RD lateral CSTs	0.0010 +/- 0.00008	0.0010 +/- 0.0001	0.45

Supplementary table 11. DTI parameters in the corticospinal tracts (CSTs) described as mean and standard deviation in C9+ subjects at baseline and at 18-month follow-up**.** Comparisons are FDR corrected and adjusted for age, gender and family membership.
C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**Baseline**	**18-months evaluation**	**p-value**
FA lateral CSTs	0.396 +/- 0.1278	0.360 +/- 0.1249	0.24
AD lateral CSTs	0.0016 +/- 0.0006	0.0015 +/- 0.0005	0.14
MD lateral CSTs	0.0006 +/- 0.0313	0.0012 +/- 0.0004	0.30
RD lateral CSTs	0.0010 +/- 0.0004	0.0110 +/- 0.0004	0.60
Subjects < 40 years	**Baseline**	**18-months evaluation**	**p-value**
FA lateral CSTs	0.389 +/- 0.1532	0.350 +/- 0.1462	0.45
AD lateral CSTs	0.0016 +/- 0.0007	0.0016 +/- 0.0006	0.78
MD lateral CSTs	0.0011 +/- 0.0004	0.0012 +/- 0.0005	0.29
RD lateral CSTs	0.0010 +/- 0.0004	0.0011 +/- 0.0005	0.28
Subjects > 40 years	**Baseline**	**18-months evaluation**	**p-value**
FA lateral CSTs	0.406 +/- 0.1090	0.368 +/- 0.1030	**0.045**
AD lateral CSTs	0.0017 +/- 0.0005	0.0015 +/- 0.0004	0.35
MD lateral CSTs	0.0098 +/- 0.0408	0.0011 +/- 0.0003	0.43
RD lateral CSTs	0.0011 +/- 0.0004	0.0009 +/- 0.0004	0.37
Subjects with a family history of ALS	**Baseline**	**18-months evaluation**	**p-value**
FA lateral CSTs	0.394 +/- 0.024	0.381 +/- 0.025	0.65
AD lateral CSTs	0.0017 +/- 0.0001	0.0015 +/- 0.00013	0.14
MD lateral CSTs	0.0098 +/- 0.0062	0.0011 +/- 0.0066	0.33
RD lateral CSTs	0.0011 +/- 0.00009	0.0010 +/- 0.0001	0.50

Supplementary table 12. DTI parameters in the dorsal columns expressed as mean and standard deviation in the entire population and in age-defined subgroups (younger and older than 40 years of age) at baseline**.** Comparisons are FDR corrected for multiple comparisons and adjusted for age, gender and family membership. C9+ = *c9orf72* positive subjects; C9- = *c9orf72* negative subjects.

Total population	**C9+**	**C9-**	**p-value**
FA dorsal columns	0.4194 +/- 0.0231	0.4309 +/- 0.0249	0.57
AD dorsal columns	0.0017 +/- 0.0009	0.0015 +/- 0.0001	0.27
MD dorsal columns	0.0012 +/- 0.0006	0.0011 +/- 0.0007	0.17
RD dorsal columns	0.0010 +/- 0.0005	0.0009 +/- 0.0006	0.18
Subjects < 40 years			
FA dorsal columns	0.3872+/- 0.0353	0.3532 +/- 0.0328	0.63
AD dorsal columns	0.0016 +/- 0.0001	0.0016 +/- 0.0001	0.85
MD dorsal columns	0.0011 +/- 0.0009	0.0012 +/- 0.0008	0.80
RD dorsal columns	0.0009+/- 0.0000	0.0010 +/- 0.0007	0.76
Subjects > 40 years			
FA dorsal columns	0.4417 +/- 0.0291	0.4523 +/- 0.0320	0.68
AD dorsal columns	0.0018 +/- 0.0001	0.0015 +/- 0.0001	0.23
MD dorsal columns	0.0012 +/- 0.0009	0.0011 +/- 0.0008	0.35
RD dorsal columns	0.0010 +/- 0.0008	0.0008 +/- 0.0007	0.13
Subjects with a family history of ALS	**C9+**	**C9-**	**p-value**
FA dorsal columns	0.4108 +/- 0.0283	0.4590 +/- 0.0336	0.30
AD dorsal columns	0.0018 +/- 0.0001	0.0017 +/- 0.0001	0.37
MD dorsal columns	0.0013 +/- 0.0007	0.0012 +/- 0.0009	0.45
RD dorsal columns	0.0010 +/- 0.0006	0.0009 +/- 0.0008	0.46

Table 13: Members of the PREV-DEMALS study group

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